IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KITAMURA=1

In re application of:	)	Art Unit: 1651
Hidetomo KITAMURA	)	Examiner: L. B. Lankford
Appln. No. 09/380,372	)	Washington, D.C.
Filed: September 1, 1999	)	
For: NOVEL CELL LINES AND SCREENING METHODS...	)	

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Hidetomo KITAMURA, a Japanese citizen, residing at 135, Komakado, 1-chome, Gotenba-shi, Shizuoka, 412-8513, Japan, hereby declare that I am the inventor of the above-entitled patent application, and that I received a D.V.M degree from Hokkaido University Faculty of Veterinary Medicine in March 1991.

I declare also that I have been employed by Chugai Seiyaku Kabushiki Kaisha, the assignee of this application, and have been engaged in pharmaceutical research since April 1991 and that I work as a researcher for Pharmaceutical Research Dept. II of Chugai Seiyaku Kabushiki Kaisha.

I also declare that I have read all of the Official Actions pertaining to the above-entitled application, and am familiar

with each of the references cited in the Official Actions by the Examiner.

I declare further that the following statements are true and correct to the best of my knowledge.

Statements

The present invention is directed to a cell line capable of differentiating into chondrocytes and capable of differentiating into adipocytes, which cell is derived from a normal adult animal. As is indicated in Example 2 of the present specification, the cell line of the present invention is capable of differentiating into chondrocytes and capable of differentiating into adipocytes in the absence of dexamethasone.

The medium used for culturing the CL-1 cells in Examples 2 and 3 of the present specification is Minimum Essential Medium a (MEMa; GibcoBRL catalogue no. 11900) containing 10% fetal calf serum (Moregate, lot no. IM1728), 100 µ/ml penicillin (Banyu Pharmaceutical Co, Ltd), and 100 µg/ml streptromycin (Meiji Seika Kaisha, Ltd). Dexamethasone was absent from the culture medium used in Examples 2 and 3 of the present specification. A copy of the pertinent pages of the 1995-1996 Gibco BRL Life Technologies Catalogue relating to MEM a medium (catalogue no. 11900) is attached hereto. It is well known that fetal calf serum and antibiotics are commonly added to medium for culturing cells.

In contrast to the presently claimed cell line, the RCJ 3.1 cells of the Grigoriadis et al. reference, J. Cell Biology, 106:2139-2151 (1988), relied upon by the examiner in the final rejections, were derived from mesenchymal cells of fetal rat calvaria and are unable to differentiate into adipocytes or chondrocyes in the absence of dexamethasone (abstract, left column; page 2142, right column, lines 1-11 of "Adipocyte Formation"; paragraph "Cartilage Formation" on bridging pages 2144 and 2145). Grigoriadis specifically teaches on page 2149, left column, that:

> We have confirmed, however, that dexamethasone is required for progenitors to become committed to and differentiate along adipocytes and chondrocyte lineages, because RCJ 3.1 cells, subcloned in the absence of dexamethasone, only produced clones which differentiated into muscle, but not into fat or cartilage (data not shown).

Worster et al., Journal of Orthopaedic Research, 19:738-749 (2001), a copy of which is attached hereto, shows that mesenchymal progenitor stem cells obtained from adult horses (3 days to 2 years old) differentiated into chondrocytes in the absence of dexamethasone (Abstract, page 738, Material and methods, pages 740-743, and Result and Discussion, pages 744-747). In the Materials and Methods section under Mesenchymal cell cultures (page 740, right column), it is taught that mesenchymal stem cells (MSCs) were cultured in serum-free Ham's F-12 medium containing the additives previously described (fetal bovine serum,

ascorbic acid, a-ketoglutaric acid, L-glutamine, sodium penicillin, streptomycin sulfate, and HEPES buffer, as disclosed in the left column of page 740) and supplemented with TGF-ß1. Ham's F-12 medium is a well known and commonly used nutrient culture medium in the art and its formulation is given in the pertinent pages of the 1995-1996 GibcoBRL Life Technologies Catalogue attached hereto. As can be clearly seen, neither the additives supplementing Ham's F-12 medium nor the formulation of Ham's F-12 medium, the defined medium used for differentiation (chondrogenesis), contain dexamethasone. Hence, dexamethasone was not required for chondrogenesis in Worster.

The results of Example 2 of the present application and the disclosure of Worster demonstrate that the cell lines derived from a normal adult animal as recited in claim 1 of the present application can differentiate into adipocytes and chondrocytes regardless of the presence or absence of dexamethasone. By contrast, dexamethasone is required for the RCJ 3.1 cells of Grigoriadis in order to differentiate into adipocytes and chondrocyes. Accordingly, the cell line of the present invention is clearly distinguishable from and patentable over the RCJ 3.1 cell line of the Grigoriadis reference.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these

statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Dated this 17 day of March 2003

Hidetomo Kitamura

Hidetomo KITAMURA

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